

EDITORIAL COMMENT

C-Reactive Protein and the Vascular Endothelium

Implications for Plaque Instability*

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C-reactive protein (CRP), a member of the pentraxin family, is the prototypic marker of inflammation. Recently data have emerged to suggest that CRP is a risk marker for cardiovascular disease and is additive to low-density lipoprotein (LDL) cholesterol and the Framingham 10-year risk score (FRS) (1). In this regard, the American Heart Association and Centers for Disease Control and Prevention have recommended that in patients with an FRS of 10% to 20%, a CRP between 3 and 10 mg/l confers a greater risk of cardiovascular disease (2). Furthermore, Grundy et al. (3) incorporated CRP >3 mg/l as part of the two risk factors in moderately high-risk patients with an FRS of 10%

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to 20% in whom a therapeutic option or an LDL cholesterol goal of <100 mg/dl should be offered. The CRP levels seem to be a good metric of genetic and environmental factors that could predispose to cardiovascular disease. These include increased adiposity, smoking, metabolic syndrome, diabetes, hypertension, chronic kidney disease, sleep apnea, and so on. Although previously it was believed that CRP was produced exclusively by the liver, recent data suggest that CRP is also produced in human atheroma. Yasojima et al. (4) provided cogent evidence showing that CRP mRNA in atheroma were ten times greater than in the normal vessel. Other groups have confirmed that CRP messenger ribonucleic acid is present in directed atherectomy samples and that smooth muscle cells and endothelial cells synthesize and secrete CRP (5,6). The obvious question that arises is whether CRP in the vessel wall can contribute to atherothrombosis and plaque instability.

The article by Montero et al. (7) in this issue of the *Journal* shows that CRP increased levels of certain matrix metalloproteinases (MMP-1 and -10) from human umbilical vein endothelial cells (HUVECs) and human aortic endothelial cells (HAECs) without any significant change

in the tissue inhibitor of MMP. They also showed that CRP treatment resulted in an increase in MMP activity. Furthermore, specific inhibition of p38 mitogen-activated protein kinase (MAPK) or MAPK-activated ERK kinase (MEK) abolished the CRP induction of MMP-1, whereas blockade of MMP-10 induction required the simultaneous blockade of p38 MAPK and Jun N-terminal kinase (JNK) pathways. In patients with CRP >3 mg/l compared with patients with lower levels of CRP, both MMP-1 and -10 levels were elevated after adjusting for confounding variables. Finally, they showed that CRP and MMP co-localize in the endothelial layer and macrophage-rich areas of advanced atherosclerotic plaques. These are important observations that support the contention that CRP is a marker of plaque activity and can indeed participate in acute coronary syndromes (ACS). However, it is disappointing that the investigators focused most of their findings in HUVEC given that aortic endothelial cells are the primary site of atherosclerosis. Their findings confirm those of a previous group that show that CRP increases production of MMP-1 in macrophages through the fragment of antibody with the c-terminal of immunoglobulin chain (Fc) gamma receptor II, CD32, via ERK activation (8). Recently, it has been shown in HAEC that CRP, via the Fc gamma receptors CD32 and CD64, mediates its biological effects (9). Thus, in the present report, the investigators should have also elucidated the receptor pathway because they show by confocal microscopy that CRP co-localizes with MMP in the endothelial layer. The data with regard to elevated MMP-1 and -10 in patients with high CRP levels cannot denote cause and effect. Previously, Lin et al. (10) showed that CRP induces MMP-2 and -9 activities in HUVEC. These collective findings suggest that CRP may promote matrix degradation and thus contribute to plaque vulnerability. The concept that CRP might participate in atherothrombosis is not new. Pasceri et al. (11) previously showed in HUVEC and human coronary artery endothelial cell (HCAEC) that CRP up-regulated vascular cell adhesion molecule (VCAM-1), intercellular adhesion molecule (ICAM-1), E-selectin, and monocyte chemoattractant protein-1 (MCP-1) and promoted increased monocyte adhesion. Two laboratories independently reported that CRP decreased endothelial nitric oxide synthase (eNOS) messenger ribonucleic acid, protein, and activity in aortic and saphenous vein endothelial cells (6). Also, CRP has been shown to inhibit prostacyclin in HAEC via nitration of prostacyclin synthase (6). Other studies that support a role for CRP in atherothrombosis include up-regulation of tissue factor procoagulant activity in monocytes, increased plasminogen activator inhibitor-1 (PAI-1) activity, and inhibition of tissue plasminogen activator (tPA) antigen and activity in HAEC (6,12). C-reactive protein stimulates PAI-1 via up-regulation of Rho kinase (13) and inhibits tPA via up-regulation of interleukin (IL)-1 and tumor necrosis factor in HAEC (12). Patients with high CRP compared with those

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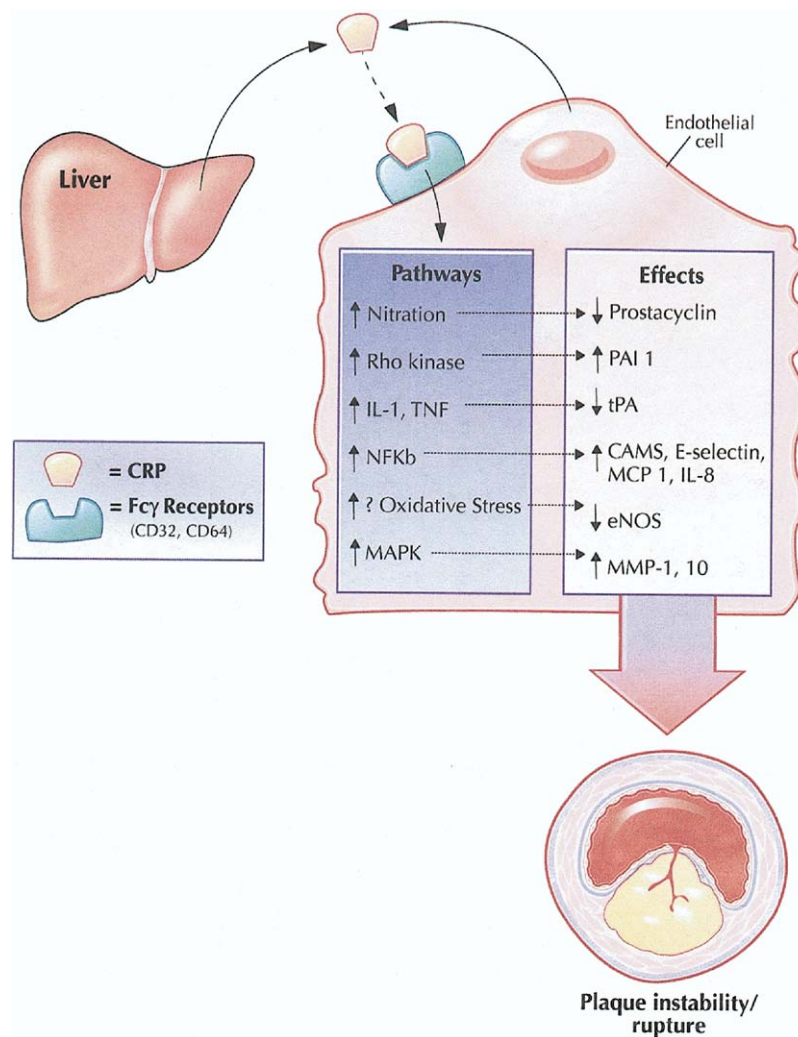


Figure 1. Effects of C-reactive protein (CRP) on the vascular endothelium. CAMS = cell adhesion molecules; eNOS = endothelial nitric oxide synthase; Fcγ = fragment of antibody with the c-terminal of immunoglobulin chain; IL = interleukin; MAPK = mitogen-activated protein kinase; MCP 1 = monocyte chemotactic protein-1; MMP-1 = matrix metalloproteinase-1; NFKb = nuclear factor kb; PAI-1 = plasminogen activator inhibitor-1; TNF = tumor necrosis factor; tPA = tissue plasminogen activator.

with low CRP had a prolonged euglobin clot lysis time (12), supporting the data in the human CRP transgenic mice showing a higher frequency of arterial thrombosis of the transgenic mice compared with wild-type mice after femoral injury (14).

Thus, CRP has numerous effects on endothelial cells that could support a pro-inflammatory, pro-thrombotic role (Fig. 1). Its procoagulant effects include inhibition of eNOS, prostacyclin, tPA and up-regulation of PAI-1, and the proinflammatory effects include up-regulation of IL-6, adhesion molecules, ICAM and VCAM, and the chemokines, MCP-1 and IL-8. It is also important to note here that the effects of CRP on eNOS, prostacyclin, tPA, PAI-1, and IL-8 have been shown to be independent of endotoxin and azide contamination as reported also by Montero et al. (7,12,13). Furthermore, in addition to stimulating tissue factor in macrophages, CRP has been shown to promote the uptake of oxidized LDL, which would be relevant to the genesis of the atherosclerotic lesion, including plaque insta-

bility (6). The recent observations that CRP is produced in aortic endothelial cells and that secreted CRP could be augmented 100-fold with human macrophage-conditioned media incubated with endothelial cells argues for paracrine and autocrine loops in the atheroma that could result in exceedingly high CRP concentrations in microdomains (5). Indeed, plasma CRP levels ranging from 20 to 64 mg/l have been reported in patients with ACS (15,16), and levels seem to be higher in aortic sinus samples and to predict poorer outcomes (17–19).

In conclusion, data are emerging to support a role for CRP in atherothrombosis; CRP has been shown to induce myocardial infarction in a rat coronary ligation model and increased cerebral infarct size in rats after middle cerebral artery occlusion (20,21). Furthermore, CRP has been shown to promote neointimal formation after balloon angioplasty (22). With regard to the data in mice, it is unclear how fruitful this line of investigation would prove to be because the major acute phase reactant in mice is not CRP, but

serum amyloid A (SAA) and serum amyloid P component (SAP). Thus it is not surprising that the studies to date that have introduced human CRP into mice have yielded conflicting results (23).

If CRP contributes to plaque instability and the genesis of ACS, then modulating CRP in the setting of ACS may prove beneficial. In this regard, exciting new data are emerging. In both Pravastatin or Atorvastatin Evaluation and Infection therapy (PROVE-IT) and the A to Z study (15,24), concomitant reduction of LDL and CRP with statin therapy resulted in a greater benefit in cardiovascular end points, further supporting the idea that CRP might be an active participant in atherothrombosis and the genesis of ACS. However, these exciting preliminary findings need to be confirmed in future studies.

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